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Commentary

HuR keeps Interferon-β mRNA stable

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Abstract

The expression of Interferon (IFN)-β is highly induced in immune and non-immune cells in response to virus infection. This upregulation is mediated at both transcriptional and posttranscriptional levels. Whereas the signaling pathways triggered by innate virus receptors leading to transcription factor activation have been extensively studied, the mechanisms by which IFN-β mRNA stability is posttranscriptionally controlled are not fully understood. In this issue of the *European Journal of Immunology*, Herdy et al. [Eur. J. Immunol. 2015 45:XXXX-XXXX] show that a human RNA-binding protein, Embryonic Lethal, Abnormal Vision, Drosophila-Like 1 (ELAVL1)/Hu antigen R (HuR), strongly associates with IFN-β mRNA via AU-rich sequences present in its 3' untranslated region in various human cell lines. The authors show that ELAVL1/HuR is required for the stabilization of IFN-β mRNA, and suppression of ELAVL1/HuR by its inhibitor MS-444 leads to impaired expression of IFN-β in response to viral dsRNA treatment. Thus, this study uncovers a novel mechanism of posttranscriptional IFN-β mRNA regulation in response to virus infection, through IFN-β stabilization by ELAVL1/HuR. Future studies are expected to identify further regulatory mechanisms of IFN-β stabilization and destabilization in the course of antiviral responses.

Keywords:

Type I Interferon, Gene regulation, Innate immunity, mRNA stability, antiviral response, Mass spectrometry, ELAVL1/HuR

See accompanying article by Herdy et al.

Main text

Type I Interferons (IFNs), comprised of multiple IFN-α isoforms and an IFN-β, are critical for innate and adaptive antiviral host defense as well as for the pathogenesis of autoimmunity [1, 2]. Secreted type I IFNs subsequently stimulate the type I IFN receptor (IFNAR) to transactivate a set of IFN-stimulated genes (ISGs) in autocrine and paracrine manners. The expression of type I IFN mRNAs is rapidly induced by the recognition of virus infection via a set of pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and viral DNA sensors [3]. TLRs are transmembrane proteins recognizing microbial components on the cell surface or in the endosomes. Among the TLRs expressed on the endosome, TLR3, TLR7 and TLR9 sense viral double-stranded (ds) RNA, single-stranded (ss) RNA and DNA with a CpG motif respectively. Other PRRs, such as the cytoplasmic RLRs, RIG-I and Melanoma differentiation-associated gene 5 (MDA5), recognize 5'-triphosphate end dsRNA and long dsRNA respectively [4]. Extensive studies have revealed the PRR signaling pathways leading to transcription of type I IFNs via transcription factors including IFN-regulatory factor (IRF) 3 and IRF7 [1]. PRR signaling also activates another transcription factor, NF-κB, which contributes to the transactivation of proinflammatory cytokines as well as IFN-β. However, posttranscriptional regulation is also important for the regulation of mRNAs for type I IFNs and proinflammatory cytokines [5, 6].

It is well known that mRNAs encoding proinflammatory cytokines and type I IFNs have short half-lives, which is understood to be important for preventing unnecessarily sustained inflammation[7]. The regulation of cytokine mRNAs such as TNF and IL-6 has been shown to be mediated by cis-acting elements present in the 3′ untranslated regions (UTRs) and the coding region, including AU-rich elements (AREs) and stem-loop structures [5-7]. AREs are known to be recognized by various

RNA-binding proteins (BPs) called ARE-BPs. Among these, tristetraprolin (TTP), ARE/poly-(U) binding degradation factor 1 (AUF1), and KH domain-splicing regulatory protein (KSRP) are reported to be involved in the destabilization of mRNAs harboring AREs (Figure 1A). For instance, TTP decreases *TNF* mRNA stability by binding to AREs present in its 3′ UTRs, leading to deadenylation by recruitment of a CCR4-NOT deadenylase complex (Figure 1A) [8, 9]. In contrast, the RNA-binding protein Embryonic Lethal, Abnormal Vision, Drosophila-Like 1 (ELAVL1)/Hu antigen R (HuR) has been shown to be involved in increasing the stability of these mRNAs [5, 10, 11]. In addition, stem-loop structures present in the 3' UTR of TNF and IL-6 mRNAs also act as cis-elements for their destabilization. For instance, stem-loop structures present in a set of mRNAs, including those encoding ICOS, OX40 and TNF, have been shown to promote mRNA instability via Roquin-1 and Roquin-2, which interact with a ROQ domain within the stem-loop [12, 13]. Furthermore, an RNase called Regnase-1 (also known as Zc3h12a) has been shown to degrade IL-6 mRNA via a stem-loop sequence present in its 3' UTR [14, 15]. The mechanisms controlling the stability of IFN-β mRNA, however, are not yet fully understood.

In this issue of the *European Journal of Immunology*, Herdy et al. first demonstrate that an ARE present in the 3' UTR of IFN-β mRNA causes the mRNA to be destabilized in HeLaS3 cells [16]. In contrast, an IFN-β mRNA destabilizing element present in the coding region instability determinant (CRID) showed only a modest effect in destabilizing IFN-β mRNA in this cell type (Figure 1B). Whereas IFN-β mRNA exhibits a relatively short half-life, the mRNA was stabilized in response to stimulation with Polyinosinic–polycytidylic acid (Poly I:C), a viral dsRNA analog recognized by TLR3 and MDA5. These results suggest that TLR and/or RLR signaling may control the stability of IFN-β mRNA via the 3' UTR.

To identify proteins interacting with the ARE, the authors combined affinity purification of ARE20 RNA, which comprises the first 20 nucleotides of the IFN-β ARE, with mass spectrometry to isolate and identify proteins interacting in this region. Among 93 proteins obtained by mass spectrometry, ELAVL1/HuR was identified as the sole ARE-binding protein associating with the IFN-β ARE (Figure 1B) [16]. In contrast, ARE-BPs known to destabilize mRNAs, such as TTP, AUF1 and KSRP, were not present in the proteins co-precipitated with the IFN-β ARE. A previous study using PAR-CLIP analysis of HuR-binding mRNA sites had revealed that HuR- and microRNA-binding sites tend to reside in proximity to each other in HEK293 cells, implying that dimerized HuR stabilizes mRNAs by preventing microRNA-mediated degradation [17]. If TTP, AUF1 and KSRP are not responsible for driving IFN-β mRNA instability, could microRNAs, then, be responsible for promoting IFN-β mRNA decay? Further studies may identify microRNA(s) responsible for the degradation of IFN-β mRNAs, which bind close to the ARE.

Although the half-life of IFN-β mRNA was short in unstimulated cells, it was dramatically stabilized following 8 hours of poly I:C stimulation [16]. Furthermore, this stabilization ceased after 16 hours of stimulation, indicating that the stability of IFN-β mRNA is tightly and transiently controlled by TLR and RLR signaling. TTP has been shown to be phosphorylated by the p38 mitogen-activated protein kinase (MAPK)-activated kinase MK2 in the course of MAP kinase signaling, which occurs downstream of stimulation by various cytokines and PRRs. TTP phosphorylation induces formation of a complex between TTP and 14-3-3, which dissociates TTP from stress granules and inhibits recruitment of the CCR4-NOT complex [18, 19]. Whereas TTP was not identified as an IFN-β ARE-BP in this study, it is possible that a different ARE-BP acting on IFN-β mRNA is modified in the course of poly I:C stimulation. Although the binding of ELAVL1/HuR to the IFN-β ARE was not altered in the course

of stimulation [16], it is interesting to speculate that the activity of ELAVL1/HuR to stabilize IFN-β mRNA is dynamically controlled by TLR and RLR signaling. Alternatively, poly I:C-triggered signaling pathways may alter the expression of a set of microRNAs controlling the stability of IFN-β mRNA. Further studies are however required to understand the molecular mechanisms controlling IFN-β mRNA stability upon virus infection.

When HuR expression was depleted with siRNA, the expression of IFN-β mRNA in response to poly I:C stimulation was severely impaired in HeLaS3 cells [16]. Furthermore, the antiviral activity of poly I:C-stimulated HeLaS3 cell supernatants was diminished in response to knockdown of ELAVL1/HuR. Therefore, HuR is critical for the expression of IFN-β mRNA and antiviral responses to TLR3 and MDA5 activation. The activity and importance of HuR in the stability of IFN-β mRNA was further confirmed by inhibition of HuR with a chemical compound, MS-444. Consistent with HuR knockdown, MS-444 treatment suppressed the expression of IFN-β in response to poly I:C stimulation in HeLaS3 cells. MS-444 also showed inhibitory effects in controlling IFN-β expression in fibroblast-like synoviocytes. Although type I IFNs are critical for protection against virus infections, their pathogenic roles in autoimmunity and bacterial infection have also been documented [2]. Therefore, suppression of IFN-β mRNA expression by inhibitors of ELAVL1/HuR might be useful for the treatment of such diseases. Given that the concentration of MS-444 required for the suppression of IFN-β mRNA is relatively high, further evaluation is required to identify more efficient small molecules for the suppression of HuR dimerization.

Herdy et al. focused on non-immune cells such as HeLaS3 and synovial fibroblast cells for assessing the requirement of ELAVL1/HuR for IFN-β mRNA expression [16]. However, it is well known that myeloid immune cells, such as

macrophages and conventional or plasmacytoid dendritic cells, are the major producers of type I IFNs in response to virus infection [20]. Therefore, it will be interesting to investigate if IFN-β mRNA stability is controlled by ELAVL1/HuR in a similar manner in these cells.

In summary, this study provides new insights into the molecular mechanisms controlling type I IFN production in response to viral infection. Future studiesmay further characterize the mechanisms and importance of posttranscriptional regulation of IFN-β in antiviral immune responses.

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Conflict of Interest

The author declares no financial or commercial conflict of interest.

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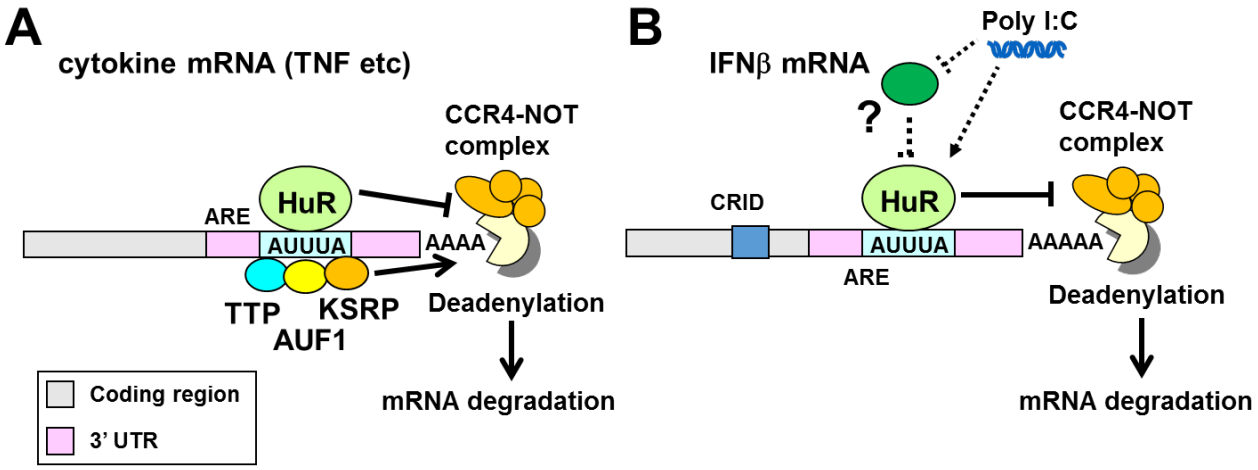

Figure 1. HuR-mediated stabilization of IFN-β mRNA via the 3' UTR.

(A) The regulation of cytokine mRNAs such as TNF has been shown to be mediated by cis-acting elements present in the 3′ untranslated regions (UTRs), including AU-rich elements (AREs), and within the coding region, known as CRID. AREs are known to be recognized by various RNA-binding proteins (ARE-BPs). Among these, tristetraprolin (TTP), ARE/poly-(U) binding degradation factor 1 (AUF1), and KH domain-splicing regulatory protein (KSRP) are reported to be involved in the destabilization of mRNAs harboring AREs. For instance, TTP decreases *TNF* mRNA stability by binding to AREs present in its 3′ UTRs, leading to deadenylation by recruitment of a CCR4-NOT deadenylase complex .

(B) IFN-β mRNA harbors two destabilization sequences, CRID and ARE. Although the known ARE-BPs driving mRNA instability were not identified by Herdy et al. [16], HuR may act to ARE-BP acting on IFN-β mRNA is modified in the course of poly I:C stimulation